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(54) Title: IMPROVED NUCLEIC ACID REACTIONS

(57) Abstract

A method of promoting a reaction between a duplex-binding ligand and a duplex nucleic acid molecule, the duplex nucleic acid molecule being formed from a first single strand nucleic acid molecule and a second single strand nucleic acid molecule, under conditions wherein the rate of duplex formation would be substantially less in the absence of the duplex-binding ligand.

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IMPROVED NUCLEIC ACID REACTIONS

This invention was made with government support under grant #GM-39471 from the National Institutes of Health. Accordingly, the U.S. Government retains certain rights in the invention.

Background of the Invention

This invention relates to the formation and dissolution of double stranded nucleic acid molecules and to the interactions between double and single stranded nucleic acid molecules and nucleic acid-binding ligands.

Reactions between duplex DNA and ligands are largely dictated and mediated by the interplay of structural, thermodynamic and dynamic characteristics of DNA, and recognition mechanisms of reacting ligands. Ligands that bind to DNA span a broad range of sizes from small cations to large proteins and assembled protein aggregates. A wide variety of experimental strategies have been employed to examine sequence specificity exhibited by ligands that interact with DNA. Sequence dependent variations in local conformation and charge configuration along DNA are thought to be the principle means by which ligands discriminate between various DNA sequences. Such discrimination can be divulged and quantitatively evaluated from sequence specific thermodynamic binding parameters evaluated in studies of ligand/DNA complex formation.

Double helical DNA structure is maintained by a number of forces. Among these are the strong Coulombic interactions between phosphates along and across the backbone, hydrogen bonding between base pairs (bps) across the helix axis, stacking interactions between bps along one strand and across the helix axis and a multiplicity of interactions with charged solvent components. Inadequate understanding of these interactions precludes the construction of a realistic atomic model that correctly simulates the helixcoil or melting transition in DNA.

The most successful analytical approaches to modeling the helix-coil transition in DNA relate to the statistical thermodynamic formalism of the modified Ising model (R.M. Wartell and A.S. Benight, Physics Rpts., 126, 67-107 (1985)). In this approach the central assumption is that each bp of a DNA helix can occupy only one of two possible states. These are the "intact" and "broken" states. In the intact state a given bp is presumed to be hydrogen bonded and completely stacked with its neighboring bps on either side. Alternatively, in the broken or melted state a bp is not hydrogen bonded and is completely unstacked from its neighbors on either side.

In most models, melting stability arises from independent contributions of individual bps. More sophisticated models consider nearest-neighbor (n-n) interactions. Comparison of actual absorbence-versus-temperature measurements (melting curves) with calculations allow

evaluation of the sequence-dependent energetics of DNA melting within the context of the two-state per bp model.

Over the past 30 years optical and calorimetric melting studies of duplex DNA have established that the melting temperature, t_m, of DNA is a linearly increasing function of the percent of the bps that are of the guanine-cytosine type (%G·C). Greater stability of DNA with increased %G·C can be most readily attributed to the fact that G·C bps, with three hydrogen bonds are more stable than A·T bps with only two hydrogen bonds. Sequence dependent stacking interactions between neighboring bps may also contribute to this difference in a minor way. Thus to first order, DNA stability can be expressed as a number-weighted sum of the individual energies of two components, these being the "energies" of A·T and G·C bps. For a specific sequence, i, this energy (the H-bond energy) can be designated:

$$\Delta G_{H\text{-}bond}(i) = \Delta S_{AT} N_{AT} T_{AT} + \Delta S_{GC} N_{GC} T_{GC} \qquad (1)$$

N_{AT} and N_{GC} are the numbers of A·T and G·C bps in the sequence and T_{AT} and T_{GC} are the average melting temperatures of A·T (T·A) and G·C (C·G) bps. Values of T_{AT} or T_{GC} evaluated from melting curve analysis of a variety of DNAs collected as a function of solvent environment provide the dependence of t_m on solvent ionic strength. The dependence of T_{AT} and T_{GC} on $[Na+]^{22}$ was first reported by M.D. Frank-Kamenetski (Biopolymers, 10, 2623-24 (1971)).

$$T_{AT} = 355.55 + 7.95 \ln\left[Na^+\right] \qquad (2a)$$

$$T_{GC} = 391.55 + 4.89 \ln\left[Na^+\right] \qquad (2b)$$

ΔS_{AT} and ΔS_{GC} in eqn (1) are the average entropy changes associated with melting A·T or G·C bps. Calorimetric and spectrophotometric melting studies of long DNA polymers of natural and synthetic origins have revealed the transition entropies of melting A·T and G·C bps are virtually in-dependent of bp type (A·T or G·C), temperature, and only weakly dependent on solvent ionic strength over reasonable limits (15 mM to 1.0 M NaCl) (Ref ?). Assuming only three preferred conformations are available for each nucleotide residue per bp, the transition entropy in forming a helix can be written as:

$$\Delta S = -2\,(6R \cdot \ln 3) = -26.2 \text{ cal} / K \cdot \text{mol} \qquad (3)$$

Coincidentally, this value is almost precisely the entropy of base pair formation, ΔS = -24.85 $\pm$ 1.84 cal/K mole, determined from the studies mentioned above. Thus, $\Delta S_{AT} = \Delta S_{GC} = \Delta S$ can be determined from the ratio:

$$\Delta H_{AT} / T_{AT} = \Delta H_{GC} / T_{GC=\Delta S} \quad (4)$$

where ΔH_{AT} and ΔH_{GC} are enthalpy changes in melting A·T or G·C bps. Calorimetric and spectrophotometric melting studies of short duplex oligomers six to eight bps in length have revealed a sequence dependence of the melting entropy (K.J. Breslauer, et al., Proc. Nat. Acad. Sci. USA, 83, 3746-50 (1986)).

The values of the bp transition enthalpies, ΔH_{AT} and ΔH_{GC}, are also dependent on solvent ionic strength. Empirically derived equations for their determination in different Na+ environments have also been reported. S.A Kozyaukin, et al., J. Biomol. Struct. Dynam., 5, 119-26 (1987).

$$\Delta H_{AT} = -9300 - 456.01 \ln[Na^+] \quad (5)$$

From eqns (2b) and (4), ΔH_{GC} can be determined. Therefore, if DNA is considered to be comprised of only two energetic components, the free-energy can be determined from the sequence by substitution of the appropriate values from eqns. 2, 4 and 5 in eqn. 1.

During the mid-70's substantial quantities of homogeneously pure DNA samples were available. In addition, spectrophotometric instrumentation allowed automated collection of melting curve data with increased resolution. These developments made possible the discovery of multi-model melting or "fine-structure" on optical melting transitions of heterogeneous-sequence DNA fragments. Such fine structure was attributed to sequential melting of large DNA domains. Failure of simple two-component melting theories to accurately predict the observed fine structure in DNA melting curves suggested a role for both sequence heterogeneity and sequence type in the transition.

The potential for bound ligands to affect the structure of unbound flanking DNA sequences has been recognized for some time (reviewed by D.M. Crothers and M. Fried, Cold Spring Harbor Symposia Quant. Biol., 47, 263-69 (1983)). Footprinting methodology has been applied to detect unbound, but structurally perturbed regions flanking a ligand binding site. The location of actinomycin D binding was monitored by the inaccessibility of DNAseI to DNA within the drug binding site. M. Lane, et al., Proc. Nat. Acad. Sci USA, 80, 3260-64 (1983); C.M.L. Low, et al., Nucl. Acids Res., 12, 4865-79 (1984). Structural perturbations imparted to flanking DNA sequences by the bound drug were simultaneously monitored as enhanced DNaseI cleavage rates at immediately flanking sequence positions not sterically occluded from DNaseI by bound drug. Although the potential of DNA structural distortions at regions within the drug footprint exist, the footprinting approach cannot detect such distortions since these regions are protected from cleavage. When intercalated at its dinucleotide site in a linear molecule, actinomycin D can affect flanking DNA structure in a linear DNA molecule over considerable distances albeit with sequence dependence. Further

corroboration that drug induced DNaseI detected enhancements were structural in origin was independently obtained from proton NMR experiments of d[(AAATATAGCTATATTT)$_2$] (SEQ ID NO: 1) complexed with actinomycin D. K.D. Bishop et al., Nucl. Acids Res., 19, 871-75 (1991).

Restriction enzymes cleave duplex DNA at specific nucleotide sequences. The sequences flanking a restriction enzyme recognition site can influence the rate of restriction enzyme cleavage at the site. M.C. Aloyo, et al., Biophys. J., 64, A280 (1993). Such effects occur while cleaving P4 phage DNA with the restriction enzyme EcoRI, suggesting that differences in DNA sequences flanking EcoRI sites account for observed differences in rates of cleavage. Goldstein, et al., Virology, 66, 420-427 (1975). A large body of data regarding the sequence-dependent behavior of various restriction enzymes has appeared. Armstrong and Bauer, Nucl. Acids Res., 11, 4109-4126 (1983), and Alves, et al., Eur. J. Biochem., 140, 83-92 (1984), disclosed cleavage rate variations for the enzymes EcoRI, HinfI, and PstI, finding that the activities of all three enzymes could be inhibited by long runs of GC-rich sequences placed immediately flanking the restriction sites. Concerning effects of flanking DNA sequence on cleavage by enzymes FnuDII, HaeIII, HhaI and MspI, Drew and Travers, Nucl. Acids Res., 13, 4445-4456 (1985), observed that cleavage rates for these enzymes exhibit a dependence on flanking sequence, noting that the effect "though clearly evident, was complex and varied."

Variations in rates of restriction enzyme cleavage have also been shown to be dependent on DNA substrate length. Thus, the rate of cleavage at a specific site depends directly on the length of DNA flanking the specific site. Richter and Eigen, Biophys. Chem., 2, 255-263 (1974); Berg, et al., Biochemistry, 20, 6929-6948 (1981).

Summary of the Invention

In general, the invention features a method of promoting a reaction between a duplex-binding ligand and a duplex nucleic acid molecule, the duplex nucleic acid molecule being formed from a first single strand nucleic acid molecule and a second single strand nucleic acid molecule, under conditions wherein the rate of duplex formation would be substantially less in the absence of the duplex-binding ligand. The method includes: forming a reaction mixture including the first single strand nucleic acid molecule, the second single strand nucleic acid molecule, and the duplex-binding ligand under conditions wherein the rate of duplex formation would be substantially less in the absence of the duplex-binding ligand, the duplex binding ligand being present at an amount, concentration, or chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of the duplex-binding ligand, thereby allowing the reaction between the duplex and the duplex binding ligand to proceed, and

reacting the duplex-binding ligand with the duplex.

In another aspect, the invention features a method of performing a polymerase reaction using as a double stranded substrate a duplex nucleic acid molecule formed from a first (e.g., a probe) and a second (e.g., a target) single strand nucleic acid molecule. The method includes:

(1) forming a reaction mixture comprising a plurality of the first single strand molecules, at least one of the second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, an amount, concentration, or chemical potential of the single strand binding ligand and an amount, concentration, or chemical potential of the duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and the second strand,

(b) a reaction between the polymerase and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and the second strand, and

(e) a reaction between the polymerase and the second formed duplex; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

In another aspect, the invention features a reaction mixture comprising a single strand nucleic acid molecule, a second single starnd nucleic acid molecule, a duplex and a duplex-binding ligand at an amount, concentration, or chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of the duplex-binding ligand.

In another aspect, the invention features a reaction mixture including: a plurality of the first single strand molecules, at least one second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, an amount, concentration, or chemical potential of the single strand binding ligand and an amount, concentration, or chemical potential of the duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and the second strand,

(b) a reaction between the polymerase and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and the second strand, and

(e) a reaction between the polymerase and the second formed duplex.

The methods and reagents of the invention can be used to determine the presence of a nucleic acid sequence present in a sample, and thus determine if a disease-related organism is

present in a sample. The invention allows more sensitive and more selective detection of the nucleic acids of disease-related organisms, e.g. viruses, bacterium (e.g., the agents responsible for septicemia), and protazoans. Additionally, methods of the invention for detection of pathogens or other organisms are extremely simple to use and in many embodiments use isothermal reactions and are particularly suted for amplification reactions for diagnostic purposes.

A variety of DNA-based amplification strategies which rely upon recognition of a primer:template duplex by a polymerizing enzyme or other polymerizing agents have been reported. Some of these methods are based on repetitive sampling of a target nucleic acid, so as to generate a signal through a linear process. Other methods are based on the exponential amplification of target DNA. Exponential methods can result in significant signal amplification but often also result in the amplification of nonspecific targets. Most traditional methods by which primers which initiate such reactions are designed are largely based on poorly-understood thermodynamic parameters and weak-to-nonexistent nucleic acid structural motif arguments. This has often led to primer design which is virtually random and which involves empirical assessment of fidelity for each primer in such a reaction. The methods of the invention provide for a fundamental understanding of current amplification/detection strategies and allow for the improvement of these methods as well as the design of new nucleic acid based amplification strategies. One basis for the improved understanding provided by the inventors derives from consideration of the fact that all DNA binding agents act as chemical reagents, as do nucleic acids.

Methods of the invention provide for nucleic acid reactions in which the nucleic acid components cycle between single strand and duplex forms. This is of particular use in reactions which depend on the presence of both single strand and duplex forms to proceed, or in reactions in which cycles of annealing and reannealing are needed to allow amplification or detection of a target nucleic acid. The cycling is thermodynamically propelled and does not require thermal cycling. Thermodynamic cycling is provided for by including in the reaction a balanced mixture of single strand and duplex nucleic acid binding ligands to insure that the reaction [single strand + single strand $\Leftrightarrow$duplex] proceeds in both directions at rates which allow the production of a significant level of a desired product. Thus, methods of the invention rely on the use of both an agent that preferentially binds single strands and an agent that preferentially binds duplex to achieve free energy balance between single strands and duplex and thereby provide thermodynamic cycling. Methods of the invention allow cycling reactions under isothermal conditions, dispensing with the need for temperature-resistant enzymes and thermal cycling which are needed in many detection schemes.

The inclusion of an appropriate level of single strand binding ligand can also result in more selective hybridization, and thus allow greater selectivity in hybridization based reactions. Thus, the invention provides methods and reagents which allow the formation of reaction mixtures in which a target nucleic acid and a probe nucleic acid complementary to

the target hybridize with high accuracy. These methods allow virtual elimination of nonhomologous, partially self complementary sequences which can hybridize to the probe (albeit in a weaker manner). Such partially homologous sequences are normally present in practical application (e.g., clinical samples) and contribute background to the detected signal.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Detailed Description

The drawings are first briefly described.

Drawings

Fig. 1 is a diagram of a generalized reaction in which the chemical potentials of a single strand binding agent and a duplex binding agent provide for cycling between single strand and duplex states.

Fig. 2 is a diagram of a polymerase chain reaction driven by net chemical potential change, also referred to as isothermal PCR.

Fig. 3 is a depiction of the products of an isothermal PCR reaction as analyzed by gel electrophoresis.

Improved Nucleic Acid Reactions

Tm, as used herein, refers to the midpoint of the duplex to single strand melting transition.

The chemical potential of a reagent , as used herein, the change in free enrgy of a reagent mix when the reagent is added to the mix. Chemical potential is a more exact measure of the activity of a species in a given reaction under a given set of conditions and takes into account considerations such as the number of sites a species can react with and whether all molecules of a species are available for reactions. Chemical potential of a species can usually be most directly manipulated by changing the concentrations of a species. The unit of chemical potential is free-energy, with units, e.g., of cal/mole or J/mole, but in the methods described herein determination of an absolute chemical potential is not required. Most of the methods disclosed herein require reaction mixtures in which the ratio of chemical potential between two species, or the difference between the chemical potential of two species, is required to be such that a given result, e.g., isotheromal cycling is achieved.

A single strand binding ligand, as used herein, refers to a ligand which preferentially binds a single strand nucleic acid (as compared to the duplex or double stranded form of the nucleic acid). Single strand binding ligands include, proteins, e.g., enzymes, as well as non-protein compounds. Single strand binding protein (SSB), the G-5 protein, the gene 32 protein, Rec A, and helicases, are examples of single strand binding ligands. Single strand binding ligands stabilize the single stranded form and can thus be identified by their effect on

the Tm of a duplex. A single strand binding ligand results in a decrease in the Tm of a duplex. Thus, duplex melting point determinations performed in the presence and absence of a test-compound will indicate if the test-compound is a single strand binding ligand for a sequence. Before use in methods of the invention, the test-compound should be tested to determine if it has any unwanted effects on other components of the reaction mix. For example, the single strand binding ligand used in DNA polymerase-dependent DNA amplification reactions should not inhibit DNA polymerase to a substantial degree. Furthermore, the term single strand binding ligand, refers both to homogenous and heterogenous ligands. A homogenous single strand binding ligand is one which includes a single chemical species. A heterogenous single strand binding ligand is one which includes more than one chemical species, e.g., one which includes a proteinaceous single strand binding ligand and a non-protenaceous single strand binding ligand. In a heterogenous single strand binding ligand the various included species can exhibit different interactions with the single strand. For example, a heterogenous single strand binding ligand can include a polymerase which interacts with the single strand by binding to it and catalyzing a covalent modification of the single strand, and a species which binds to the single strand but which does not catalyze a covalent modification of the single strand.

A double strand or duplex binding ligand, as used herein, refers to a ligand which preferentially binds the double strand or duplex form of a nucleic acid in preference to single strands of the nucleic acid . Double strand binding ligands can be proteins, e.g., enzymes, or non-protein compounds. DNA polymerase, actinomycin, and daunomycin are examples of double strand binding ligands. Double strand binding ligands stabilize the duplex form and can thus be identified by their effect on the Tm of a duplex. A double strand binding ligand results in an increase in the Tm of a duplex. Thus, duplex melting point determinations performed in the presence and absence of a test-compound will indicate if the test-compound is a double strand binding ligand for a sequence. Before use, the test-compound should be tested to determine if it has any unwanted effects on other components of the reaction mix. Furthermore, the term duplex binding ligand, refers both to homogenous and heterogenous ligands. A homogenous duplex binding ligand is one which includes a single chemical species. A heterogenous duplex binding ligand is one which includes more than one chemical species, e.g., one which includes a proteinaceous duplex binding ligand and a non-protenaceous dup;exbinding ligand. In a heterogenous duplex binding ligand the various included species can exhibit different interactions with the duplex. For example, a heterogenous duplex binding ligand can include a polymerase which interacts with the duplex by binding to it and catalyzing the formation of a new strand, and a species which binds to duplex but which does not catalyze the formation of a new strand.

Reaction, as used herein, refers to an interaction in which there is and alteration of the structure of the nucleic acid including ,e.g.: breaking or forming a covalent or non-covalent bond, e.g., a hydrogen bond, between an atom of a nucleic acid molecule and another atom or

between two atoms of the nucleic acid, cleaving of one or both strands of the nucleic acid; catalyzing the synthesis of a nucleic acid, e.g., catalyzing the synthesis of DNA from a double stranded section formed by the hybridization of the first sequence to the second, e.g., performing a DNA polymerase catalyzed reaction, e.g., DNA polymerase I or Taq polymerase catalyzed reaction; altering the primary or secondary structure of the nucleic acid; catalyzing or promoting the conversion of single strands to duplex; catalyzing or promoting the conversion of duplex to single strands.

Purified nucleic acid, as used herein, refers to a purified DNA or RNA. Purified DNA, as used herein, refers to DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the DNA is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Purified RNA, as used herein, refers to an RNA which is substantilally free of another RNA sequence with which it is found in a cell wich produces the RNA.

Purified natural product, as used herein, is a product which is produced by an organism and which is substantially free of a macromolecule, e.g., a protein or a nucleic acid, with which it occurs in an organism for m which it is derived.

A product which does not naturally occur in living cells, as used herein, refers to a product which is not synthesized or produced by living cells or organisms.

In general, the invention features a method of promoting a reaction between a duplex-binding ligand and a duplex nucleic acid molecule, the duplex nucleic acid molecule being formed from a first single strand nucleic acid molecule and a second single strand nucleic acid molecule, under conditions wherein the rate of duplex formation would be substantially less in the absence of the duplex-binding ligand. The method includes: forming a reaction mixture including the first single strand nucleic acid molecule, the second single strand nucleic acid molecule, and the duplex-binding ligand under conditions wherein the rate of duplex formation would be substantially less in the absence of the duplex-binding ligand, the duplex binding ligand being present at an amount, concentration, or chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of the duplex-binding ligand, thereby allowing the reaction between the duplex and the duplex binding ligand to proceed, and

reacting the duplex-binding ligand with the duplex.

In preferred embodiments: the method is performed under isothermal conditions; the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of duplex formation in the absence of duplex-binding ligand is substantially zero; at least one of the strand molecules

is a purified nucleic acid molecule; the duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potential in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the duplex from the first and the second sequence is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the duplex-binding-ligand to the reaction mix; the ratio by weight, molarity, number, concentration, or chemical potential of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a

reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further includes detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase.

In another aspect, the invention features a method of promoting a reaction between a duplex-binding ligand in a reaction mix and a duplex nucleic acid molecule, the duplex nucleic acid molecule formed from a first and a second single strand nucleic acid molecule, at a temperature substantially greater than the Tm of the duplex in the absence of the duplex-binding ligand. The method includes: forming a reaction mixture comprising the first single strand nucleic acid molecule, the second single strand nucleic acid molecule, and the duplex-binding ligand at a temperature substantially greater the Tm of the duplex in the absence of the duplex-binding ligand, the duplex binding ligand being present at an amount, concentration, or chemical potential which results in the formation of a duplex at the temperature, which thereby allows the reaction between the duplex and the duplex binding ligand to proceed, and

reacting the duplex-binding ligand with the duplex at the temperature.

In preferred embodiments: the method is performed under isothermal conditions; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of duplex formation in the absence of duplex-binding ligand is substantially zero; at least one of the strand molecules is a purified nucleic acid molecule; the duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the duplex from the first and the second sequence is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the duplex-binding-ligand to the reaction mix; the ratio by weight, molarity, or number of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction

mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further includes detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase.

In another aspect, the invention features a method of performing a cycling reaction between a duplex binding ligand and a duplex nucleic acid molecule formed from a first (e.g. a probe) and a second (e.g. a target) single strand nucleic acid molecule. The method includes:

(1) forming a reaction mixture comprising a plurality of the first single strand molecules, at least one of the second single strand molecules, a single strand binding ligand, and a duplex strand binding ligand, the chemical potential of the single strand binding ligand and the chemical potential of the duplex strand binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first strand and the second strand,

(b) a reaction between the duplex binding ligand and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and a the second strand, and

(e) a reaction between the duplex binding ligand the second formed duplex; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present,

or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single

strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal amount, concentration, or chemical potential in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex - binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA

polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments the method further includes detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase; the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a method of performing a polymerase reaction using as a double stranded substrate a duplex nucleic acid molecule formed from a first (e.g., a probe) and a second (e.g., a target) single strand nucleic acid molecule. The method includes:

(1) forming a reaction mixture comprising a plurality of the first single strand molecules, at least one of the second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, an amount, concentration, or chemical potential of the single strand binding ligand and an amount, concentration, or chemical potential of the duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and the second strand,

(b) a reaction between the polymerase and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and the second strand, and

(e) a reaction between the polymerase and the second formed duplex; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product DEFINE; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of

molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the single strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal an amount, concentration, or chemical potential in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule.

In other preferred embodiments: the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex -binding-ligand to the single strand in the

highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand, in addition to a polymerase, comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the duplex binding ligand is a DNA polymerase; the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a method of cyclically reacting a duplex binding ligand with a duplex formed by a hybridization of a first single strand molecule (e.g., a probe) molecule to a second single strand molecule (e.g., a target). the method includes:

forming a reaction mixture comprising the first single strand molecule, the second single strand molecule, a single strand binding ligand, and a duplex binding ligand, the chemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of the single strand binding ligand being such that duplex dissociation reaction occurs concurrently with the formation reaction, the chemical potentials being such that the rate of duplex formation and the rate of duplex dissociation are such that a cycle of (duplex binding ligand:duplex binding)-(duplex dissociation)-(duplex binding ligand:duplex binding)could occur under isothermal conditions, and

allowing the cycle to proceed.

In other preferred embodiments: the cycle can occur least n times under isothermal conditions, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: wherein the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the

second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the cyclic reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase; the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a method of detecting the hybridization of a first single strand molecule (e.g., a probe) molecule to a second single strand molecule (e.g., a target) comprising:

forming a reaction mixture comprising the first molecule, the second molecule, a single strand binding ligand, and a duplex binding ligand comprising a polymerase, a ligase, or other sequence forming enzyme, an amount, concentration, or chemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and an amount, concentration, or chemical potential of the single strand binding ligand being such that a predetermined level of duplex dissociation reaction occurs, the rate of duplex formation and the rate of duplex dissociation being such that a cycle of duplex binding ligand catalyzed formation-duplex dissociation-duplex binding ligand catalyzed formation which can occur under isothermal conditions occurs,

allowing the reaction to proceed in the presence of any cofactor or substrate required by the duplex binding ligand, e.g., NTPs, and

detecting the hybridization of the first molecule to the second molecule by detecting the formation of a sequence by the duplex binding ligand.

In other preferred embodiments: the single strand molecule is labeled with a detectable marker when it hybridizes to the second strand; the single strand molecule is labeled after hybridizing to the second molecule; the NTPs are labeled and a labeled extension product is detected; the single strand binding ligand is a purified natural product or a non-naturally occurring product DEFINE; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another

atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal an amount, concentration, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex - binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the duplex binding ligand; the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a method of promoting a reaction between a single-strand-binding ligand and a single-strand nucleic acid molecule, the single strand nucleic acid molecule being formed from a duplex comprising the first single strand nucleic acid molecule and a second single strand nucleic acid molecule, under conditions wherein the rate of single-strand formation would be substantially less in the absence of the duplex-binding ligand. The method includes:

forming a reaction mixture comprising the first single strand nucleic acid molecule, the second single strand nucleic acid molecule, and the single strand-binding ligand under conditions wherein the rate of single strand formation would be substantially less in the absence of the single-strand-binding ligand, the single strand binding ligand being present at a chemical potential which results in the formation of a single strand at a rate which is substantially greater than the rate in the absence of the single strand-binding ligand, thereby allowing the reaction between the single strand and the single strand binding ligand to proceed, and

reacting the single strand-binding ligand with the single strand.

In other preferred embodiments: the method is performed under isothermal conditions; the rate of single strand formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of single strand binding ligand; the rate of single strand formation in the absence of single strand-binding ligand is substantially zero; at least one of the single strand molecules is a purified nucleic acid molecule; the single strand binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the single strand-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the single strand from the duplex is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the single strand-binding-ligand to the reaction mix; the ratio by weight, molarity, number, concentration, or chemical potential of the single strand-binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a the ligand; a compound which, when contacted with a reaction mixture comprising a duplex of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand

molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions; the product is a nucleic acid sequence the formation of which is catalyzed in the reaction mix by the single strand binding ligand.

In another aspect, the invention features a method of promoting a reaction between a single strand-binding ligand in a reaction mix and a single strand nucleic acid molecule, the single strand nucleic acid molecule formed from a duplex comprising a first and a second single strand nucleic acid molecule, at a temperature substantially less than the Tm of the duplex in the absence of the single strand-binding ligand. The method includes:

forming a reaction mixture comprising the first single strand nucleic acid molecule, the second single strand nucleic acid molecule, and the single strand-binding ligand at a temperature substantially less the Tm of the duplex in the absence of the duplex-binding ligand, the single strand binding ligand being present at an amount, concentration, or chemical potential which results in the formation of a single strand at the temperature, which thereby allows the reaction between the single strand and the single stand binding ligand to proceed, and

reacting the single strand-binding ligand with the single strand at the temperature.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of single strand formation in the absence of single strand-binding ligand is substantially zero; at least one of the strand molecules is a purified nucleic acid molecule; the single strand binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the single strand-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration,

number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the single strand from the duplex is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the single strand-binding-ligand to the reaction mix; the ratio by weight, molarity, number, or concentration of chemical potential of the single strand-binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a single strand nucleic acid to which is binds; an enzyme which alters the structure of a single strand nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves the nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target

sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions.

In another aspect, the invention features a method of performing a cycling reaction between a single strand binding ligand formed from a duplex nucleic acid molecule comprising a first (e.g. a probe) and a second (e.g. a target) single strand nucleic acid molecule comprising:

(1) forming a reaction mixture comprising a plurality of the first single strand molecules, at least one of the second single strand molecules, a single strand binding ligand, and a duplex strand binding ligand, an amount, concentration, or chemical potential of the single strand binding ligand and an amount, concentration, or chemical potential of the duplex strand binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the disassociation of a first duplex between an initial first strand and the second strand,

(b) a reaction between the single strand binding ligand and the initial first strand,

(c) the formation of a second duplex between a subsequent first single strand and a the second strand, and

(d) the disassociation of the second formed duplex,

(e) a reaction between the single strand binding ligand the subsequent first strand; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product DEFINE; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another

atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is below the Tm of the duplex; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal an amount, concentration or chemical potential in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the single strand -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex formed of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand

molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions.

In another aspect, the invention features a method of cyclically reacting a single strand binding ligand with single strand formed from a duplex formed by a hybridization of a first single strand molecule (probe) molecule to a second single strand molecule (target) including:

forming a reaction mixture comprising the first single strand molecule, the second single strand molecule, the single strand binding ligand, and a duplex binding ligand, the chemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of the single strand binding ligand being such that duplex dissociation reaction occurs concurrently with the formation reaction, the chemical potentials being such that the rate of duplex formation and the rate of duplex dissociation are such that a cycle of (duplex dissociation)-(single strand binding ligand:single strand binding)-(duplex formation) could occur under isothermal conditions, and

allowing the cycle to proceed.

In other preferred embodiments: the cycle can occur least n times under isothermal conditions, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a

restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the method is performed under isothermal conditions; the temperature is below the Tm of the duplex; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; at least one of the strand molecules is a purified nucleic acid molecule; the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first

single strand and the second single strand, the duplex, or the combination of the first single strand, the66

second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the single strand -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex formed of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid,

e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the method further including detecting a product of the reactions.

In another aspect, the invention features a reaction mixture comprising a single strand nucleic acid molecule, a second single strand nucleic acid molecule, a duplex and a duplex-binding ligand at an amount, concentration, or chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of the duplex-binding ligand.

In other preferred embodiments: the duplex binding ligand is present at an amount, concentration, or chemical potential sufficient that the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of duplex formation in the absence of duplex-binding ligand is substantially zero; at least one of the strand single molecules is a purified nucleic acid molecule; the duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the duplex binding ligand is present at an amount, concentration, or chemical potential sufficient that wherein the free energy for the formation of the duplex from the first and the second sequence is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the duplex-binding-ligand to the reaction mix; the ratio by weight, molarity, number, concentration, or chemical potential of the duplex -binding-ligand to the single strand in the

highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase.

In another aspect, the invention features a reaction mixture including: a duplex-binding ligand; a first single strand nucleic acid molecule, a second single strand nucleic acid

molecule, the duplex binding ligand being present at an amount, concentration, or chemical potential which results in the formation of a duplex at a temperature substantially above the Tm of the duplex of the first and second strands.

In other preferred embodiments: the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; the duplex binding ligand is present at a chemical potential sufficient that wherein the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of duplex formation in the absence of duplex-binding ligand is substantially zero; at least one of the strand molecules is a purified nucleic acid molecule; the duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the duplex binding ligand is present at an amount, concentration, or chemical potential sufficient that wherein the free energy for the formation of the duplex from the first and the second sequence is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the duplex-binding-ligand to the reaction mix; the ratio by weight, molarity, or number of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a

restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In another aspect, the invention features a reaction mixture comprising: a plurality of first single strand molecules, at least one second single strand molecules, a single strand binding ligand, and a duplex strand binding ligand, an amount, concentration, or chemical potential of the single strand binding ligand and the chemical potential of the duplex strand binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first strand and the second strand,

(b) a reaction between the duplex binding ligand and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and a the second strand, and

(e) a reaction between the duplex binding ligand the second formed duplex.

In other preferred embodiments: the cycle can occur at least n times, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product DEFINE; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the strand molecules is a purified nucleic acid molecule; the number of molecules of, or an amount, concentration, or chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or an amount, concentration, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to

which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase; the single strand binding ligand is present in sufficient concentration, number of molecules, or an amount, concentration, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In other preferred embodiments a reaction mixture including: a plurality of the first single strand molecules, at least one second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, an amount, concentration, or chemical potential of the single strand binding ligand and an amount, concentration, or chemical potential of the duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and the second strand,

(b) a reaction between the polymerase and the first formed duplex,

(c) the dissolution of the first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and the second strand, and

(e) a reaction between the polymerase and the second formed duplex.

In other preferred embodiments: the cycle occurs at least n times, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential

of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the single strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal an amount, concentration, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex-binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand, in addition to a polymerase, comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid

to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the duplex binding ligand is a DNA polymerase.

In other preferred embodiments: the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a reaction mixture including: a duplex binding ligand; a first single strand molecule, a second single strand molecule, a single strand binding ligand, and a duplex binding ligand, the chemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of the single strand binding ligand being such that duplex dissociation reaction occurs concurrently with the formation reaction, the chemical potentials being such that the rate of duplex formation and the rate of duplex dissociation are such that a cycle of (duplex binding ligand:duplex binding)-(duplex dissociation)-(duplex binding ligand:duplex binding)could occur under isothermal conditions.

In other preferred embodiments: the cycle can occur least n times under isothermal conditions, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand includes any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules

present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the temperature is above the Tm; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a

nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the duplex binding ligand is a polymerase, e.g., a DNA polymerase, or a ligase.

In other preferred embodiments: the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a reaction mixture including: a first single strand molecule; a second single strand molecule;

a single strand binding ligand, and a duplex binding ligand comprising a polymerase, a ligase, or other sequence forming enzyme, the chemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of the single strand binding ligand being such that a predetermined level of duplex dissociation reaction occurs, the rate of duplex formation and the rate of duplex dissociation being such that a cycle of duplex binding ligand catalyzed formation-duplex dissociation-duplex binding ligand catalyzed formation which can occur under isothermal conditions.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the

concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the cycle can occur above the Tm of the duplex; the cycle can occur at at least n C° above the Tm of the duplex, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80; at least one of the strand molecules is a purified nucleic acid molecule; the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal an amount, concentration, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule.

In other preferred embodiments: the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the ratio by weight, molarity, number, concentration, or chemical potentials of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the duplex binding ligand comprises any of: a compound which binds to a duplex nucleic acid in a sequence-specific way; a compound which binds to a duplex nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a duplex nucleic acid to which is binds; an enzyme which alters the structure of a duplex nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves one or both strands of a duplex nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the duplex to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of

a nucleic acid; a nucleic acid polymerase; a nucleic acid polymerase which requires a double stranded primer; a DNA polymerase; DNA polymerase I; Taq polymerase; an RNA polymerase; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the duplex bound by a ligand; a compound which intercalates into a double stranded nucleic acid; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of duplex formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In other preferred embodiments: the single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of the first single strand to a sequence other than the second single strand.

In another aspect, the invention features a reaction mixture including: a single-strand-binding ligand; a first single strand nucleic acid molecule; a second single strand nucleic acid molecul; and a duplex-binding ligand,

the single strand binding ligand being present at a chemical potential which results in the formation of a single strand at a rate which is substantially greater than the rate in the absence of the single strand-binding ligand, thereby allowing the reaction between the single strand and the single strand binding ligand to proceed.

In other preferred embodiments: the rate of single strand formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of single strand binding ligand; the rate of single strand formation in the absence of single strand-binding ligand is substantially zero; at least one of the single strand molecules is a purified

nucleic acid molecule; the single strand binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the single strand-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix.

In other preferred embodiments: the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the single strand from the duplex is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the single strand-binding-ligand to the reaction mix; the ratio by weight, molarity, number, concentration, or chemical potential of the single strand-binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a the ligand; a compound which, when contacted with a reaction mixture comprising a duplex of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In another aspect, the invention features a reaction mixture including: a first single strand nucleic acid molecule; a second single strand nucleic acid molecule, and a single strand-binding ligand, the single strand binding ligand being present at a chemical potential which results in the formation of a single strand at a temperature substantially less than the Tm of the duplex.

In other preferred embodiments: the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand; the rate of single strand formation in the absence of single strand-binding ligand is substantially zero; at least one of the strand molecules is a purified nucleic acid molecule; the single strand binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

In other preferred embodiments: the concentration, number of molecules of, or the chemical potential of, the single strand-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the free energy for the formation of the single strand from the duplex is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of the single strand-binding-ligand to the reaction mix; the ratio by weight, molarity,

number, or concentration of chemical potential of the single strand-binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a single strand nucleic acid to which is binds; an enzyme which alters the structure of a single strand nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves the nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of duplex formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In another aspect, the invention features a reaction mixture including: a plurality of first single strand molecules, at least one of the second single strand molecules, a single strand binding ligand, and a duplex strand binding ligand, the chemical potential of the single strand binding ligand and the chemical potential of the duplex strand binding ligand being such that the following cycle of events can occur at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or 10^6 under isothermal conditions,

(a) the disassociation of a first duplex between an initial first strand and the second strand,

(b) a reaction between the single strand binding ligand and the initial first strand,

(c) the formation of a second duplex between a subsequent first single strand and a the second strand, and

(d) the disassociation of the second formed duplex,

(e) a reaction between the single strand binding ligand the subsequent first strand.

In other preferred embodiments: the single strand binding ligand is a purified natural product or a non-naturally occurring product; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand includes any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of

molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the temperature is below the Tm of the duplex; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive; the ratio by weight, molarity, number, concentration, or chemical potentials of the single strand -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a

compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex formed of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

In another aspect, the invention features a reaction mixture including: a first single strand molecule, a second single strand molecule, a single strand binding ligand, and a duplex binding ligand, an amount, concentration, orchemical potential of the duplex binding ligand being sufficient to cause the formation of duplex, and an amount, concentration, or chemical potential of the single strand binding ligand being such that duplex dissociation reaction occurs concurrently with the formation reaction, an amount, concentration, or chemical potentials being such that the rate of duplex formation and the rate of duplex dissociation are such that a cycle of (duplex dissociation)-(single strand binding ligand:single strand binding)-(duplex formation) could occur under isothermal conditions.

In other preferred embodiments: the cycle can occur least n times under isothermal conditions, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6; the single

strand binding ligand is a purified natural product or a non-naturally occurring product the concentration, number of molecules present, or chemical potential of the single strand binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which it binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which methylates the nucleic acid to which it binds; an enzyme which alkylates the duplex nucleic acid to which it binds; an enzyme which alters the primary or secondary structure of a duplex nucleic acid to which it binds; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by a ligand; a compound which, when contacted with a reaction mixture comprising a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the single strand binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the duplex binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no more than 10 times the concentration, number of molecules present, or chemical potential of the single strand binding ligand; the concentration, number of molecules present, or chemical potential of the duplex binding ligand is no less than one tenth the concentration, number of molecules present, or chemical potential of the single strand binding ligand.

In other preferred embodiments: the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is substantially equal to the rate of formation of single strands; the

concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of duplex is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of single strands; the concentration, number of molecules present, or chemical potential of the duplex binding ligand and the concentration, number of molecules present, or chemical potential of the single strand binding ligand are such that the rate of formation of single strands is no more than 2, 5, 10, 20, 50, 100, 10^3 or 10^4 times the rate of formation of duplex.

In other preferred embodiments: the temperature is below the Tm of the duplex; the temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, below the Tm; at least one of the strand molecules is a purified nucleic acid molecule.

In other preferred embodiments: the number of molecules of, or the chemical potential of, the duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of the first single strand, the second single strand, both the first single strand and the second single strand, the duplex, or the combination of the first single strand, the second single strand, and the duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

In other preferred embodiments: the first single strand molecule and the single strand molecule are present in substantially equal amounts or at substantially equal chemical potentials in the reaction mix; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule; the first single strand molecule is a probe or primer molecule, the second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potentials of the first sequence to the second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the ratio by weight, molarity, number, concentration, or chemical potentials of the single strand -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

In other preferred embodiments: the single strand binding ligand comprises any of: a compound which binds to a single strand nucleic acid in a sequence-specific way; a compound which binds to a single strand nucleic acid in a sequence-non-specific way; a protein; an enzyme; an enzyme which alters the structure of a nucleic acid to which is binds; an enzyme which alters the structure of a nucleic acid to which it binds by breaking or forming a covalent or non-covalent bond, between an atom of the nucleic acid and another atom; an enzyme which cleaves a nucleic acid to which it binds; a restriction enzyme; a restriction endonuclease; an enzyme which alkylates the duplex nucleic acid to which it binds; a nucleic acid ligase, e.g., DNA ligase; an enzyme which promotes or catalyzes the synthesis of a nucleic acid; an enzyme which alters the primary or secondary structure of a

duplex nucleic acid to which it binds; a topoisomerase; an enzyme which promotes or inhibits recombination; a DNA binding ligand; a mutagen; a compound which enhances the expression of a gene under the control of the nucleic acid bound by the ligand; a compound which, when contacted with a reaction mixture comprising a duplex formed of a first single stranded nucleic acid and a second single stranded nucleic acid will accelerate the rate of single strand formation at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6; a compound which, when contacted with a reaction mixture, will decrease the free energy of single strand formation by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6.

In other preferred embodiments: one or both single strand molecules are DNA; one or both single strand molecules are RNA; one single strand molecule is RNA and the other is DNA; one single strand molecule is a single stranded probe and the other single strand molecule is a target sequence which is to be cleared, detected, or amplified; one single strand molecule is a synthetic, purified natural, genetically engineered, or recombinant DNA or RNA molecule, and the other single strand molecule is a naturally occurring nucleic acid, e.g., a genomic molecule, or chromosome, e.g., a viral, bacterial, plant, or animal nucleic acid; one single strand molecule is a probe and one single strand molecule is the target sequence to be detected or amplified in an amplification reaction, or in a reaction in which amplification or detection is based on the amplification or detection of the probe or a fragment of the probe.

Methods of the invention allow: detection of one target molecule in a biological sample; detection of a target strand at a concentration of 10^{-5} pMole or less; detection of a target strand at a concentration of 10^{-6} pMole or less; detection of a target strand at a concentration of 10^{-7} pMole or less; detection of a target strand at a concentration of 10^{-8} pMole or less; detection of a target strand at a concentration of 10^{-9} pMole or less; detection of a target strand at a concentration of 10^{-10} pMole or less; detection of a target strand at a concentration of 10^{-11} pMole or less; detection of a target strand at a concentration of 10^{-12} pMole or less.

Reaction mixtures of the invention include those in having: a target strand concentration of 10^{-5} pMole or less; a target strand concentration of 10^{-6} pMole or less; a target strand concentration of 10^{-7} pMole or less; a target strand concentration of 10^{-8} pMole or less; a target strand concentration of 10^{-9} pMole or less; a target strand concentration of 10^{-10} pMole or less; a target strand concentration of 10^{-11} pMole or less; a target strand concentration of 10^{-12} pMole or less.

Thermodynamic Cycling

Fig. 1 illustrates a generalized reaction in which the chemical potentials of a single strand binding agent and a duplex binding agent provide for cycling between single strand and duplex states. Complementary single stranded nucleic acids, S_1 and S_2, react, reversibly,

to form a duplex nucleic acid, D. This reaction is referred to herein as the basic reaction. The free energy for this reaction is designated ΔG_D.

If agents which preferentially bind single strand nucleic acids (SS-B) are present, then additional free energy terms (for the reaction wherein S_1 (or S_2) reacts with SS-B to form S_1:SS-B (or S_2:SS-B)) contribute to the left hand side of the basic reaction. The free energies of binding SS-B to S_1 or S_2 are given by ΔG_{SS-B}. Although for simplicity only one SS-B, which binds both S_1 and S_2, is depicted in Fig. 1, the methods and principles disclosed herein also apply to situations in which there are separate SS-B's specific for each of S_1 and S_2, or in which only a single SS-B which binds only one of the single strand species.

When agents that preferentially bind duplex (DS-B) are present, an additional free energy (for the reaction wherein DS-B reacts with D to form D:DS-B) contributes to the right hand or (duplex) side of the basic reaction. The binding to a duplex of a preferential duplex binding entity, DS-B, has a free energy ΔG_{DS-B}. The DS-B may have some affinity for single strand nucleic acid but its affinity for the duplex species must be greater.

The balance between ΔG_{SS-B} and ΔG_{DS-B} determines the net direction of the basic reaction. If these two free energies are comparable in value, then an equilibrium situation prevails under which thermodynamic cycling occurs. To provide for thermodynamic cycling the chemical potentials of SS-B and DS-B are chosen such that ΔG_{SS-B} and ΔG_{DS-B} are substantially similar in value.

The arrow on the right in Fig. 1 indicates that the chemical potential of the SS-B drives duplex form to the single strand form, completing a loop in the cycle.

The chemical potentials of SS-B and DS-B in a reaction mix will vary with each application but can be chosen empirically. The ratios of concentrations of single strand to double strand binding ligand can be determined empirically. For example, the concentrations which give thermodynamic cycling can be determined by forming a reaction mixture which includes the single strand species, the duplex, any other necessary reagents (e.g., NTP's in the case of a polymerase catalyzed amplification reaction), and either, the single strand binding ligand, or the duplex binding ligand, at a predetermined concentration. The other binding ligand is then added at progressively increasing (or decreasing) concentrations until thermodynamic cycling is achieved. If thermodynamic cycling does not occur the starting concentration of the first binding ligand can be changed and the other binding ligand then added at progressively increasing (or decreasing) concentrations until thermodynamic cycling is achieved. Thermodynamic cycling can be recognized by an increase in the reduction of the desired reaction products. The increase is often dramatic and can be 10 to 100 fold or more over what is produced in a non-cycling state. In many cases, chemical potentials wherein the single strand binding ligand is 10x above or below that of the double strand binding ligands (or vice versa) will result in thermodynamic cycling. Once a range of concentration ratios

which allows thermodynamic cycling has been identified the concentration of SS-B can be varied to provide for optimal hybridization specificity (as disclosed below).

Isothermal Amplification Reactions

Application of the methods and principles described in the previous section allow for cycling reactions to be performed under isothermal conditions. For example by providing SS-B and DS-B of the appropriate chemical potentials numerous cycles of a polymerase chain reaction can be performed under isothermal conditions. Fig. 2 illustrates a polymerase chain reaction driven by net chemical potential change, also referred to as isothermal PCR. In this example, single strand primers bind to denatured duplex target to form primer-template complexes (duplexes), with free energy ΔG_D. In the presence of DNA polymerase, which is a duplex binding agent, polymerase-duplex complexes form in a reaction with free energy $\Delta G_{D\text{-}E}$. Likewise, if single strand binding agents (SS-B) are present, primers and denatured target duplex are bound by those agents to form SS-B-primer complexes and SS-B-target complexes in reactions with free energies $\Delta G_{P:SS\text{-}B}$ and $\Delta G_{T:SS\text{-}B}$, respectively. As with the more general relationship depicted in Fig. 1, the amount of product ultimately formed depends critically on the balance between $\Delta G_{D\text{-}B}$ and $\Delta G_{T:SS\text{-}B}$.

The absolute chemical potential of the two different nucleic acid binding ligands bound with their substrates is very important in the reactions. The actual value for the duplex formation free energy ΔG_D is unimportant in the scheme if the chemical potential of each of the ligands for their substrates is high enough to promote duplex -single strand interchange. The arrow on the right in Fig. 2 indicates that the chemical potential of the SS-B drives duplex form to the single strand form, completing a loop in the cycle.

If the chemical potential of SS-B and DNA polymerase are substantially similar, theremodynamic cycling will occur, i.e., single strand primers and target molecules will cycle between the single strand and duplex states. As strands cycle between single strand and duplex repeated cycles of [primer to target binding (and thus duplex formation), polymerase binding duplex, polymerase catalyzed synthesis of new nucleic acid, and duplex melting] will occur, propelled not by thermal cycling but by thermodynamic cycling.

Although polymerase is a DS-B other species of DS-B can be added to the reaction mix to increase the chemical potential of DS-B in the reaction mix. DS-B's which do not exhibit substantial inhibition of polymerase can be used.

Control of the Specificity of Hybridization

If the polymerase or duplex:polymerase complex concentration or chemical potential is too large, the reaction will proceed so vigorously as to produce nonspecific probe:target complexes. Alternatively, if the polymerase or duplex:polymere complex concentration or chemical potential is too low, no reaction occurs. That is, the enzyme concentration

determines duplex binding and net extent of the reaction, and thereby behaves as a chemical reagent.

Standard hybridization methods, e.g., the PCR method described above, do not provide for control over accuracy, because the reaction depends only on the concentrations of the single enzyme (polymerase) present. Duplex formation can be modulated by inclusion of a single strand binding agent (SS-B). This affects the left side of the reaction by undergoing a binding reaction with the probe (to form SSB:probe) or the target (to form SSB-target) thereby contributing a free energies of single-strand ligand complex $\Delta G_{P:SS-B}$ and $\Delta G_{T:SS-B}$. As in the previous examples, the free energy difference between $\Delta G_{D:E}$ and $\Delta G_{T:SS-B}$ and $\Delta G_{P:SS-B}$ controls the direction of the cycling equilibrium by mediating duplex formation. Note that if under the conditions in which the experiment is performed the free energies $\Delta G_{D:E}$ and ΔG_{SS-B}, $\Delta G_{T:SS-B}$ for the opposing binding reactions are greater than the free energy of duplex formation, ΔG_D, only the concentrations of duplex binding agent, e.g., polymerase, and SS-B, determine the net direction of the reaction. The chemical potential of SS-B can be chosen to control the fidelity of duplex formation reactions. Fidelity of the reactions is independent of initial total DNA concentration when the duplex binding and single stand binding agents are present at high enough chemical potential. A balance between a desired level of fidelity (selectivity) and sensitivity can be provided by choosing the appropriate concentrations of chemical potentials of duplex binding agents and SS-B. These values are determined empirically or by other methods known in the art. Methods employed for describing such compositions are well known in the art.

The following examples are provided to illustrate particular embodiments of the invention.

Example 1: Isothermal PCR

The ratio of Taq polymerase (the duplex binding ligand) and single stranded binding protein (the single strand binding ligand), were chosen empirically so as to allow thermodynamic cycling in the amplification reaction described below. The single strand binding protein in the reaction also serves as the reagent which facilitates thermodynamic cycling of the system. General reaction conditions were as follows. Each tube contained: 20ng target DNA (300 bp) and primers (10pM) [one for each strand at 10pM for 100uL] in a buffer composed of 100mM TRIS-HCl, pH 7.8; $MgCl_2$ at 2.5mM; 0.2mM dNTPs; and the enzymes Taq polymerase and SSB. The ratio of Taq polymerase to SSB varied as follows: Lane 1: 0.5 units Taq polymerase, 0ug/100uL SSB; Lane 2: 0.5 units Taq polymerase, 0.25µg/100uL SSB; Lane 3: 0.5 units Taq polymerase, 1.25ug/100uL SSB; Lane 4: 0.5 units Taq polymerase, 6.25ug/100uL SSB; Lane 5: 2.5 units Taq polymerase, 0ug/100uL SSB; Lane 6: 2.5 units Taq polymerase, 0.25ug/100uL SSB; Lane 7: 2.5 units Taq polymerase, 1.25ug/100uL SSB; Lane 8: 2.5 units Taq polymerase, 6.25ug/uL SSB; Lane 9: 12.5 units Taq polymerase, 0ug/100uL SSB; Lane 10: 12.5 units Taq polymerase, 0.25ug/100uL SSB;

Lane 11: 12.5 units Taq polymerase, 1.25ug/100uL SSB; Lane 12: 12.5 units Taq polymerase, 6.25ug/100uL SSB. As can be seen from Fig. 3, at the appropriate ratios of SSB to Taq polymerase, amplification of the target DNA occurred under isothermal conditions, while no specific amplification was detectable in the absence of SSB or inappropriate ratios of SSB. In lane 9, the level of Taq polymerase in the reaction mix was so high that it drove non-specific DNA synthesis.

Example 2: Improved Ligase Chain Reaction

The ligase chain reaction can be improved by application of methods of the invention. The basic ligase chain reaction involves two probes with sequences that are complementary to adjacent positions in the target. The ligase reaction probes and denatured target form a probe-probe:target duplex with a free energy ΔG_D. Agents which preferentially bind to single strands and agents which preferentially bind to duplex can be added to cause thermodynamic cycling and to optimize selectivity. The ligase can serve as the duplex binding ligand. Ligase forms a complex with available probe:target duplex, with free energy of $\Delta G_{D:E}$. In addition, the single strand binding ligand forms complexes with the probe and target with free energies $\Delta G_{P:SS-B}$ and $\Delta G_{T:SS-B}$. The actual concentrations of these two enzymes are arrived at empirically and are such that both the forward and the backward reactions proceed with substantially similar rates under prevailing total DNA concentrations. As in previous examples, the balance between $\Delta G_{P:SS-B}$, $\Delta G_{T:SS-B}$ and $\Delta G_{D:E}$ dictate processivity of both the forward and backward reactions and effect thermodynamic cycling of the system. Upon hybridization to the target DNA ligase forms a phospodiester bond between the 3' - terminal of the first probe and the 5' - terminal of the second (adjacent) probe. The ligated probes are distinguished from the unligated probes on the basis of size.

Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK
(B) STREET: SUNY PLAZA, BROADWAY
(C) CITY: ALBANY
(D) STATE: NEW YORK
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 12210
(G) TELEPHONE:

(ii) TITLE OF INVENTION: IMPROVED NUCLEIC ACID REACTIONS

(iii) NUMBER OF SEQUENCES: 1

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII text

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 16-JUN-1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/153,535
(B) FILING DATE: 17-NOV-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/078,759
(B) FILING DATE: 17-JUN-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Paul Louis Myers
(B) REGISTRATION NUMBER: 35,965
(C) REFERENCE/DOCKET NUMBER: TMI-001CPPC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 227-4951

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAATATAGCT ATATTTAAAT ATAGCTATAT TT 32

What is claimed is:

1. A method of promoting a reaction between a duplex-binding ligand and a duplex nucleic acid molecule, said duplex nucleic acid molecule being formed from a first single strand nucleic acid molecule and a second single strand nucleic acid molecule, under conditions wherein the rate of duplex formation would be substantially less in the absence of said duplex-binding ligand comprising:

forming a reaction mixture comprising said first single strand nucleic acid molecule, said second single strand nucleic acid molecule, and said duplex-binding ligand under conditions wherein the rate of duplex formation would be substantially less in the absence of said duplex-binding ligand, said duplex binding ligand being present at a chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of said duplex-binding ligand, thereby allowing said reaction between said duplex and said duplex binding ligand to proceed, and

reacting said duplex-binding ligand with said duplex.

2. The method of claim 1, where said method is performed under isothermal conditions.

3. The method of claim 1, wherein the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, or 10^6, by the addition of duplex binding ligand.

4. The method of claim 1, wherein the rate of duplex formation in the absence of duplex-binding ligand is substantially zero.

5. The method of claim 1, wherein at least one of said strand molecules is a purified nucleic acid molecule.

6. The method of claim 1, wherein said duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

7. The method of claim 1, wherein the concentration, number of molecules of, or the chemical potential of, said duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of said first single strand, said second single strand, both said first single strand and said second single strand, said duplex, or the combination of said first single strand, said second single strand, and said duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

8. The method of claim 1, wherein said first single strand molecule and said single strand molecule are present in substantially equal amounts or at substantially equal concentration, number, or chemical potentials in said reaction mix.

9. The method of claim 1, wherein said first single strand molecule is a probe or primer molecule, said second single strand molecule is a target molecule,

10. The method of claim 1, wherein said first single strand molecule is a probe or primer molecule, said second single strand molecule is a target molecule and the ratio by weight, molarity, number, concentration, or chemical potential of said first sequence to said

second sequence is less than or equal to 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

11. The method of claim 1, wherein the free energy for the formation of said duplex from said first and said second sequence is decreased by at least n-fold, wherein n is 2, 5, 10, 50, 100, 500, 1,000, by addition of said duplex-binding-ligand to said reaction mix.

12. The method of claim 1, wherein the ratio by weight, molarity, number, concentration, or chemical potential of the duplex -binding-ligand to the single strand in the highest concentration is greater than 1:1, 2:1, 5:1, 10:1, 25:1, 50:1, 100:1, or 10^n:1, wherein n is an integer between 3 and 10, inclusive.

13. The method of claim 1, further comprising detecting a product of said reactions.

14. The method of claim 13, wherein said product is a nucleic acid sequence the formation of which is catalyzed in said reaction mix by said duplex binding ligand..

15. A method of performing a cycling reaction between a duplex binding ligand and a duplex nucleic acid molecule formed from a first (e.g. a probe) and a second (e.g. a target) single strand nucleic acid molecule comprising:

(1) forming a reaction mixture comprising a plurality of said first single strand molecules, at least one of said second single strand molecules, a single strand binding ligand, and a duplex strand binding ligand, the chemical potential of said single strand binding ligand and the chemical potential of said duplex strand binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first strand and said second strand,

(b) a reaction between said duplex binding ligand and said first formed duplex,

(c) the dissolution of said first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and a said second strand, and

(e) a reaction between said duplex binding ligand said second formed duplex; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

16. The method of claim 15, where said method is performed under isothermal conditions.

17. The method of claim 15, wherein said temperature is above said Tm.

18. The method of claim 15, wherein said temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above said Tm.

19. The method of claim 15, wherein at least one of said strand molecules is a purified nucleic acid molecule.

20. The method of claim 15, wherein said first single strand molecule is a probe or primer molecule, said second single strand molecule is a target molecule.

21. The method of claim 15, further comprising detecting a product of said reactions.

22. The method of claim 21, wherein said product is a nucleic acid sequence the formation of which is catalyzed in said reaction mix by said duplex binding ligand.

23. The method of claim 21, wherein said single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of said first single strand to a sequence other than said second single strand.

24. A method of performing a polymerase reaction using as a double stranded substrate a duplex nucleic acid molecule formed from a first (e.g., a probe) and a second (e.g., a target) single strand nucleic acid molecule comprising:

(1) forming a reaction mixture comprising a plurality of said first single strand molecules, at least one of said second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, the chemical potential of said single strand binding ligand and the chemical potential of said duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and said second strand,

(b) a reaction between said polymerase and said first formed duplex,

(c) the dissolution of said first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and said second strand, and

(e) a reaction between said polymerase and said second formed duplex; and

(2) allowing at least n, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6, of the above described cycles to occur.

25. The method of claim 24, where said method is performed under isothermal conditions.

26. The method of claim 24, wherein said temperature is above said Tm.

27. The method of claim 24, wherein said temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above said Tm.

28. The method of claim 24, wherein at least one of said single strand molecules is a purified nucleic acid molecule.

29. The method of claim 24, wherein the number of molecules of, or the chemical potential of, said duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of said first single strand, said second single strand, both said first single strand and said second single strand, said duplex, or the combination of

said first single strand, said second single strand, and said duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

30. The method of claim 24, further comprising detecting a product of said reactions.

31. The method of claim 30, wherein said product is a nucleic acid sequence the formation of which is catalyzed in said reaction mix by said duplex binding ligand.

32. The method of claim 24, wherein said single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of said first single strand to a sequence other than said second single strand.

33. A method of cyclically reacting a duplex binding ligand with a duplex formed by a hybridization of a first single strand molecule (probe) molecule to a second single strand molecule (target) comprising:

forming a reaction mixture comprising said first single strand molecule, said second single strand molecule, a single strand binding ligand, and a duplex binding ligand, the chemical potential of said duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of said single strand binding ligand being such that duplex dissociation reaction occurs concurrently with said formation reaction, said chemical potentials being such that said rate of duplex formation and said rate of duplex dissociation are such that a cycle of (duplex binding ligand:duplex binding)-(duplex dissociation)-(duplex binding ligand:duplex binding)could occur under isothermal conditions, and

allowing the cycle to proceed.

34. The method of claim 33, wherein said cycle can occur least n times under isothermal conditions, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

35. The method of claim 33, where said method is performed under isothermal conditions.

36. The method of claim 33, wherein said temperature is above said Tm.

37. The method of claim 33, wherein said temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above said Tm.

38. The method of claim 33, wherein at least one of said strand molecules is a purified nucleic acid molecule.

39. The method of claim 33, wherein the number of molecules of, or the chemical potential of, said duplex-binding ligand is greater than the concentration, number of molecules present, or chemical potential of said first single strand, said second single strand, both said first single strand and said second single strand, said duplex, or the combination of said first single strand, said second single strand, and said duplex, e.g., at least n fold greater, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

40. The method of claim 33, further comprising detecting a product of said cyclic reactions.

41. The method of claim 33, wherein said single strand binding ligand is present in sufficient concentration, number of molecules, or chemical potential such that there is substantially no non-specific hybridization of said first single strand to a sequence other than said second single strand.

42. A method of detecting the hybridization of a first single strand molecule molecule to a second single strand molecule comprising:

forming a reaction mixture comprising said first molecule, said second molecule, a single strand binding ligand, and a duplex binding ligand comprising a polymerase, a ligase, or other sequence forming enzyme, the chemical potential of said duplex binding ligand being sufficient to cause the formation of duplex, and the chemical potential of said single strand binding ligand being such that a predetermined level of duplex dissociation reaction occurs, said rate of duplex formation and said rate of duplex dissociation being such that a cycle of duplex binding ligand catalyzed formation-duplex dissociation-duplex binding ligand catalyzed formation which can occur under isothermal conditions occurs,

allowing the reaction to proceed in the presence of any cofactor or substrate required by said duplex binding ligand, e.g., NTPs, and

detecting the hybridization of said first molecule to said second molecule by detecting the formation of a sequence by said duplex binding ligand.

43. The method of claim 42, where said method is performed under isothermal conditions.

44. The method of claim 42, wherein said temperature is above said Tm.

45. The method of claim 42, wherein said temperature is at least n C°, wherein n is 5, 10, 20, 30, 40, 50, 60, 70, or 80, above said Tm.

46. The method of claim 42, wherein at least one of said strand molecules is a purified nucleic acid molecule.

47. A reaction mixture comprising a single strand nucleic acid molecule, a second single strand nucleic acid molecule, a duplex and a duplex-binding ligand at a chemical potential which results in the formation of a duplex at a rate which is substantially greater than the rate in the absence of said duplex-binding ligand.

48. The reaction mixture of claim 47, wherein said duplex binding ligand is present at a chemical potential sufficient that the rate of duplex formation is increased by at least n fold, wherein n is 2, 5, 10, 50, 100, 500, 10^3, 10^4, 10^5, 10^6, by the addition of duplex binding ligand.

49. The reaction mixture of claim 47, wherein at least one of said strand single molecules is a purified nucleic acid molecule.

50. The reaction mixture of claim 47, wherein said duplex binding ligand is a purified natural product or a product which does not naturally occur in the living cells.

51. A reaction mixture comprising: a plurality of said first single strand molecules, at least one second single strand molecules, a single strand binding ligand, and a duplex binding ligand comprising polymerase, the chemical potential of said single strand binding ligand and the chemical potential of said duplex binding ligand being such that the following cycle of events can occur under isothermal conditions,

(a) the formation of a first duplex between an initial first single strand and said second strand,

(b) a reaction between said polymerase and said first formed duplex,

(c) the dissolution of said first formed duplex,

(d) the formation of a second duplex between a subsequent first single strand and said second strand, and

(e) a reaction between said polymerase and said second formed duplex.

52. The method of claim 51, wherein said cycle occurs at least n times, wherein n is at least 1, 2, 5, 10, 25, 50, 100, 500, 10^3, 10^4, 10^5 or, 10^6.

53. The reaction mixture of claim 51, wherein said single strand binding ligand is a purified natural product or a non-naturally occurring product.

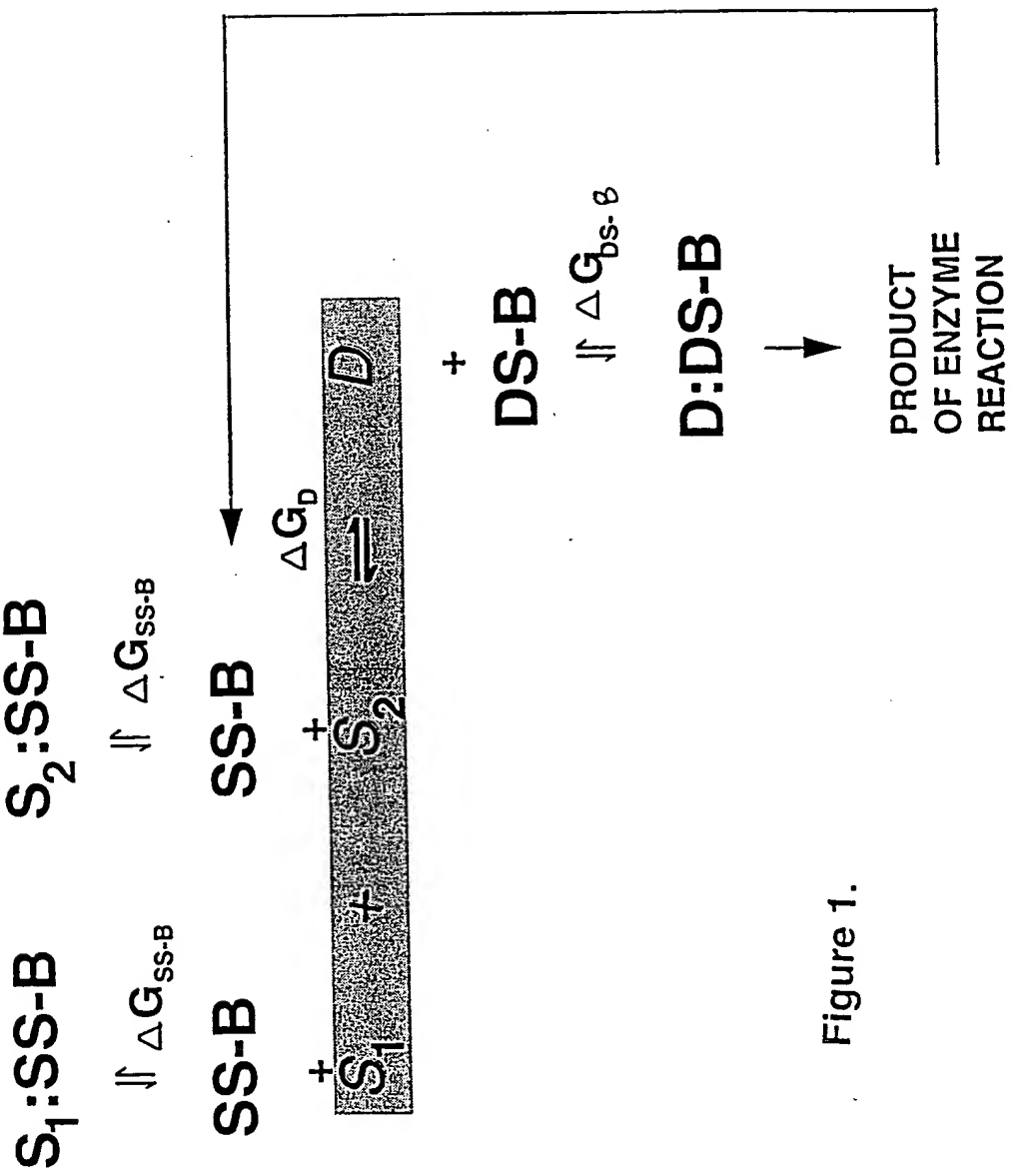
S1:SS-B
S2:SS-B
⇓ ΔGSS-B
⇓ ΔGSS-B
SS-B
SS-B
ΔGD
+ S1 + S2 D
+
DS-B
⇓ ΔGDS-B
D:DS-B
PRODUCT OF ENZYME REACTION

Figure 1.

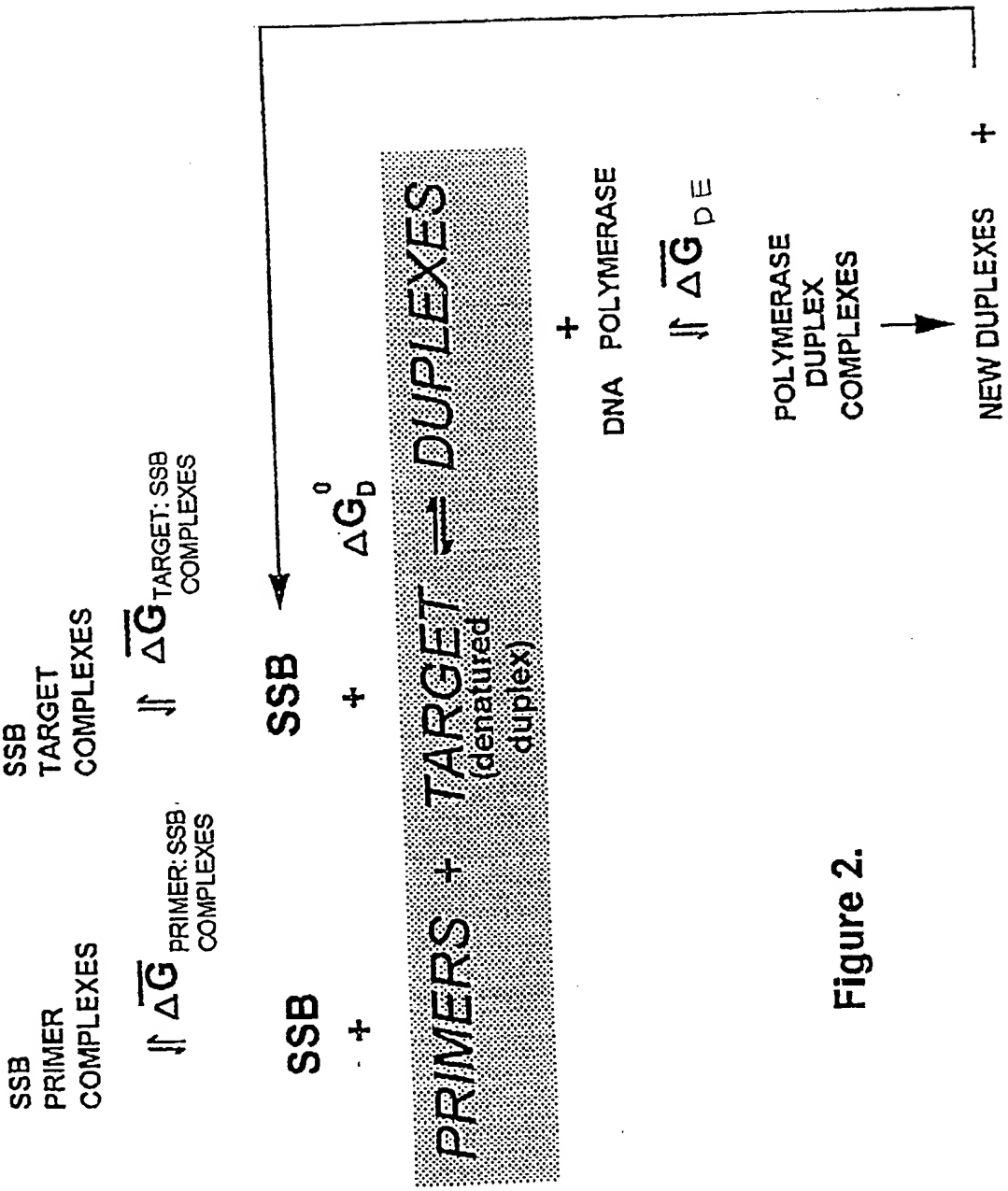
SSB
PRIMER
COMPLEXES
SSB
TARGET
COMPLEXES
PRIMER: SSB COMPLEXES
TARGET: SSB COMPLEXES
SSB
SSB
PRIMERS + TARGET (denatured duplex) DUPLEXES
DNA POLYMERASE
POLYMERASE DUPLEX COMPLEXES
NEW DUPLEXES

Figure 2.

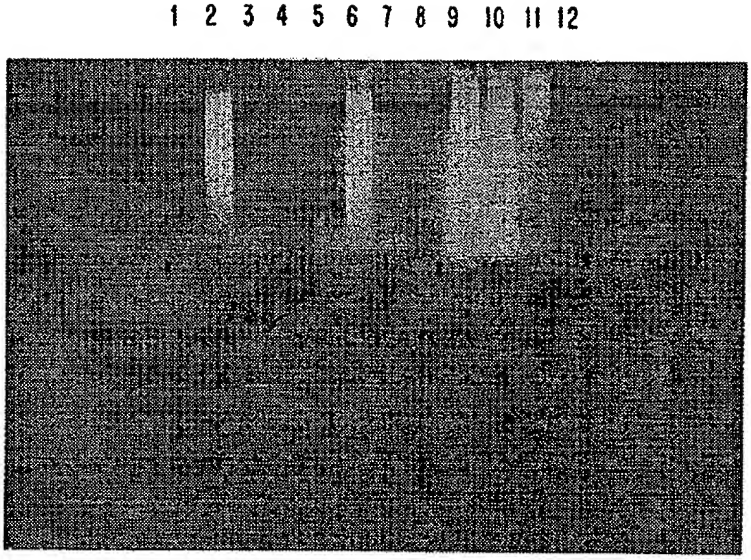

FIG. 3

INTERNATIONAL SEARCH REPORT

Intr tional application No.
PCT/US94/06800

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; C12P 19/34
US CL :435/6, 91.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,011,769 (DUCK ET AL) 30 April 1991, see entire document.	1-53

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 AUGUST 1994	Date of mailing of the international search report AUG 29 1994
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